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Male Golden Hamster in Male Reproductive Toxicology Testing: Assessment of Protective Activity of Selenium in Acute Cadmium Intoxication

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The golden hamster has a short history as a laboratory animal. In spite of this, it has been extensively used as a subject for biomedical research. The hamster has also been utilized in toxicological evaluations, especially in teratology studies. Results of these investigations reveal that laboratory hamsters are very sensitive to many chemical compounds, including: drugs, food additives, industrial chemicals, heavy metals, and other environmental contaminants (Newcomer et al. 1987). The animals most frequently used in toxicological investigations are rats and mice. This is also true in male reproductive toxicology. Apparent differences in species sensitivity to chemical compounds suggest a need to examine a new species in this field of toxicology. A good example of chemical specific differences in species sensitivity is the testicular toxicity of 1,2-dibromo-3-chloropropane (DBCP), which was a testicular toxicant in humans and in rats, but it was not effective, even at relatively high dose levels, in the mouse (Lamb IV 1988).

From our own vast experience in using hamsters in toxicological studies, we decided to use this laboratory animal in male reproductive toxicology screening tests. The purpose of this study was to determine the suitability of golden hamsters as an experimental animal species for male reproductive toxicology testing. To this effect we have chosen selenium and cadmium as test agents as they were well known for their spectacular effect on the male reproductive system.

MATERIALS AND METHODS

Mature male golden hamsters, 14-16 weeks old, derived from our own inbred colony (National Veterinary Research Institute, Pulawy, Poland) were used in this study. They were housed 5 per cage in a light controlled room (Light : Dark cycle 14:10 hr) at 20±2°C and relative humidity 50±5%. Commercial pelleted diet and water were provided *ad libitum*. The hamsters were randomly divided into four groups of 15 animals each and were dosed once subcutaneously (sc) with:

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Group I. Control (*aqua pro injectione)*
Group II. 0.5 Se mg/kg body weight (Na_2SeO_3)
Group III. 0.5 Cd mg/kg body weight ($CdCl_2$ •2 1/2 H_2O)
Group IV. 0.5 Cd mg/kg + 0.5 mg Se/kg simultaneously
The water solutions of cadmium and selenium were given in volume 0.5 ml/100 g body weight.

At 1, 4 and 10 week after treatment, 5 males at each dose were weighed and sacrificed with ethyl chloride. The corresponding spermatogenic stages at this sampling times in the hamsters were mature spermatozoa, differentiating spermatocytes and undifferentiated spermatogonia (Clermont and Trott 1969; Ghosal and Murkherjee 1971). The testes, epididymides and accessory sex glands (prostate and seminal vesicles) were removed and weighed. The right cauda epididymidis was separated, weighed and minced in 15 ml of phosphate buffered saline (PBS) with 10% formalin. The resulting suspension was diluted with PBS in the ratio of 1:10 v/v, then it was vigorously vortexed. One part of the suspension (1 ml) was treated with 10 µl of 0.025% trypsin and was used for determination of sperm number using a hemocytometer (Kempinas and Lamano-Carvalo 1988). The second one was stained with 1% Eosine Yellowish in PBS, after 30 min smears were prepared on glass slides, allowed to air dry and then flushed with distilled water. About 1000 sperm per cauda were evaluated for head shape abnormalities at 500 magnification using a green filter according to Singh classification (Singh et al 1986). Testes were fixed in 10% buffered formalin and prepared for histological examination. Sections of thickness 4-5 µm were stained with hematoxylin and eosin (HE).

The normally distributed data were analyzed by the two-way ANOVA. To determine the significance of treatment effects, Tukey and Scheffe tests were used. The abnormal sperm morphology data were analysed using a Kolmogorov-Smirnov test. The level of significance for all comparisons was set at $p<0.05$.

RESULTS AND DISCUSSION

The results are summarized in Table 1. In group III (Cd), macroscopic evaluation revealed pathological changes in the male reproductive system. In wk 1 testes, the weight of epididymides and accessory sex glands were slightly decreased when compared to controls and in the testes subcapsular hemorrhages were observed. Atrophy of the organs mentioned above gradually intensified to the end of the experiment (10 wk). There were no macroscopic pathological changes in the reproductive system in groups II (Se), IV (Se+Cd) and I (control).

The mean testes weight in the control group was 3.01 g. In Se and Cd+Se groups respective values were slightly higher. Significantly lower weight of testes was noted in the Cd group, especially at 10 wk (2.7% of the control). During the entire duration of the experiment in the Cd group,

the aforementioned organs had decreased weights.

Table 1. Effect of selenium and cadmium administered separately or simultaneously on male reproductive system

Dose mg/kg b.w.	Weeks after treatment		
	1	4	10
	testes weight (g)		
Control	3.18±0.39	2.89±0.15	2.95±0.30
Se 0.5	3.07±0.08	3.19±0.12	3.26±0.08
Cd 0.5	1.82±0.20*	0.30±0.10*	0.08±0.01*
Cd0.5 +Se0.5	3.07±0.12	3.34±0.09	2.99±0.13
	epididymis weight (mg)		
Control	521±33	548±55	592±57
Se 0.5	679±26*	702±32*	690±22
Cd 0.5	341±46*	87±12*	105±11*
Cd0.5+Se0.5	602±26	688±27	619±66
	accessory sex organs weight (g)		
Control	1.99±0.06	1.73±0.17	1.97±0.11
Se 0.5	1.97±0.15	1.86±0.20	2.04±0.08
Cd0.5	1.55±0.12	0.54±0.20*	0.90±0.18*
Cd0.5+Se0.5	2.23±0.07	2.06±0.09	1.82±0.10
	sperm number / 1 mg of cauda epididymidis (x 10^6)		
Control	0.832±0.023	0.916±0.027	0.812±0.065
Se 0.5	0.870±0.037	0.845±0.058	0.894±0.060
Cd 0.5	0.422±0.065*	0.000	0.000
Cd0.5+Se0.5	0.821±0.071	0.916±0.053	0.780±0.075
	abnormal sperm (%)		
Control	0.76±0.07	0.72±0.04	0.74±0.09
Se 0.5	0.72±0.11	0.62±0.11	0.64±0.15
Cd 0.5	0.76±0.05	n.t.	n.t.
Cd0.5+Se0.5	0.70±0.07	0.58±0.09	0.70±0.08

The values are presented as the mean ± standard error of the mean
* - significantly different, $p<0.05$
n.t. - not tested (azoospermia)
Organ weights are given per 100 g of body weight.

The mean epididymides weight in the control group was 544 mg and somewhat higher in Se and Cd+Se groups. In group III, Cd produced a marked depression in the weight of the epididymides. The lowest value in this group (15.9% of the control) was noted at 4 wk.

The mean weight of accessory sex glands in the Cd group reached 0.54 g at 4 wk, and was about four times lower than in the control animals. Values obtained in hamsters receiving either Se or Cd+Se were very similar to control.

Sperm numbers were calculated per 1 mg cauda epididymidis. In the control group, the mean caudal sperm count was 0.853×10^6. Caudal sperm count was significantly reduced (almost 6 times) after 1 wk in animals treated with Cd. At 4 and 10 wk, all males in this group showed azoospermia. The results obtained in hamsters injected with Se and Se+Cd were similar to the control group.

The percentages of sperm abnormalities were in all treatment groups very similar and did not rise above 0.8%. In the Cd group, the percentage of sperm abnormalities was not assessed because of azoospermia at 4 and 10 wk.

Histological evaluation of testes in the Cd treatment group showed serious pathological changes. After 1 wk, blood-vessels of the testes were filled, and single parenchymatous hemorrhages were observed. Except for the tunica albuginea, the whole parenchymal tissue of the testis was necrotic. Sperm cells were seen only in small numbers seminiferous tubules.

After 4 wk necrosis of the testes was still maintained and from the tunica albuginea to the center of the testis, there was an expansion of the connective tissue. Small focuses of calcinosis were also observed.

After 10 wk, the growth of connective tissue (*regeneratio incompleta*) included a whole section of the testes and the focuses of calcinosis had joined to each other.There were no pathological changes in the testes of control animals and those which received Se or Se+Cd.

Among the various organs that are known to be affected by cadmium, injury to the testes occurs at a dose which is not toxic to other organs (Wong and Klaassen 1980). In our experiment, the Cd dose 0.5 mg/kg b.w. produced hemorrhages in the testes of hamsters, followed by necrosis and finally, complete permanent testicular atrophy. Accessory sex glands and epididymides also exhibited severe atrophy. The male hamsters became infertile by the fourth week (*azoospermia*). In mice (more often used in male reproductive toxicology tests) more than twice the dose was required to cause the same pathological changes in the testes (Gunn 1965). This suggests that the male reproductive system of hamsters is more susceptible to chemical compounds than that of mice.

Selenium has a paradoxical position in animal nutrition since it is well established both as a natural toxicant and as an essential micro nutrient. Recent evidence indicates that selenium may be important for normal reproductive function in the males (Bartle et al. 1980; Shamberger 1983). Selenium had a slightly positive influence on male reproductive system in our studies. In the hamsters receiving 0.5 mg kg b.w. of Se, the weight of the testes, epididymides, accessory sex glands and sperm number were higher than those observed in the control group, but these differences were not statistically significant.
Selenium also has been shown to have a protective effect against the acute cadmium toxicity in rats (Niewenhuis et al. 1978; Ohta 1985). Ohta and Imamiya (1986) reported, that testicular damage induced by cadmium (2.0 mg/kg, ip) was protected by simultaneous administration of selenium (0.6 mg/kg, ip) in Wistar rats. Gasiewicz and Smith (1976) have suggested, that selenium interacts with plasma cadmium in a ratio of 1:1 to form a protein bond complex. In our studies, one group of hamsters simultaneously received Cd and Se in the same ratio of 1:1 (0.5 mg Cd and 0.5 mg Se per kg of body weight). In this group, selenium completely protected the male reproductive system against cadmium induced damage.

Results obtained in these experiments indicate that the male reproductive system of hamsters is susceptible to both cadmium and selenium toxicity. Moreover, this species of laboratory animal showed low percentage of spontaneous sperm abnormalities (0.74%) and a small variation in reproductive indices (weight of male reproductive organs and sperm number). In conclusion, we believe that the golden hamster may be a useful animal model in male reproductive toxicology testing.

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